IITRI

[redacted]

Report No. IITRI-L6023-2
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and Space
Administration

IIT RESEARCH INSTITUTE

Report No. IITRI-L6023-2
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

May 15 to August 15, 1965

National Aeronautics and Space Administration

Contract No. NASr-22
IITRI Project L6023

I. INTRODUCTION

Studies of the effects of different freeze cycles on Bacillus cereus and Bacillus subtilis were completed during this quarter. The results indicated that extreme diurnal temperature fluctuations cannot be regarded as a protective guarantee against contamination.

Other studies were concerned with the possible anaerobic sporulation of B. cereus and vegetative cell growth and sporulation of B. subtilis in the simulated Martian environment by using potassium nitrate as a terminal electron acceptor in place of oxygen. There were indications that B. subtilis could be adapted to anaerobic growth in the presence of nitrate by repeated transfers. Sporulation with either organism did not occur.

II. EXPERIMENTAL PROCEDURES

The simulated Martian atmosphere described in Report No. IITRI-C194-5 was used. The methods of inoculating and sampling Mars tubes also have been described in previous reports.

III. RESULTS AND DISCUSSION

Growth response and spore production data on *B. cereus* and *B. subtilis* in a simulated Martian environment for 56 days with different freeze-thaw cycles are presented in Figures 1 through 6. There were three groups:

(1) Control Group. No previous contact with the Martian environment; with 3 freeze cycles: 8, 16, and 20 hr.

(2) Transfer Group 1. Spores derived from the 8-hr freeze cycle of the Control Group after 7 days in the simulated Martian environment; with 2 freeze cycles for *B. cereus* (8 and 16 hr) and 3 freeze cycles for *B. subtilis* (8, 16, and 20 hr).

(3) Transfer Group 2. Spores derived from the 8-hr freeze cycle of Group 1 after 7 days in the simulated Martian environment; with 2 freeze cycles for *B. cereus* (8 and 16 hr) and 3 freeze cycles for *B. subtilis* (8, 16, and 20 hr).

Figure 1 presents data on the *B. cereus* Control Group. As the time of the freeze cycle was extended, the growth response was delayed. Compared to the 8-hr freeze cycle a 16-hr freeze cycle delayed spore germination and vegetative cell growth at least 2 days; sporulation was delayed as much as 4 days. Extension of the freeze cycle to 20 hr delayed spore germination and vegetative cell growth at least 6 days, and sporulation did not occur.

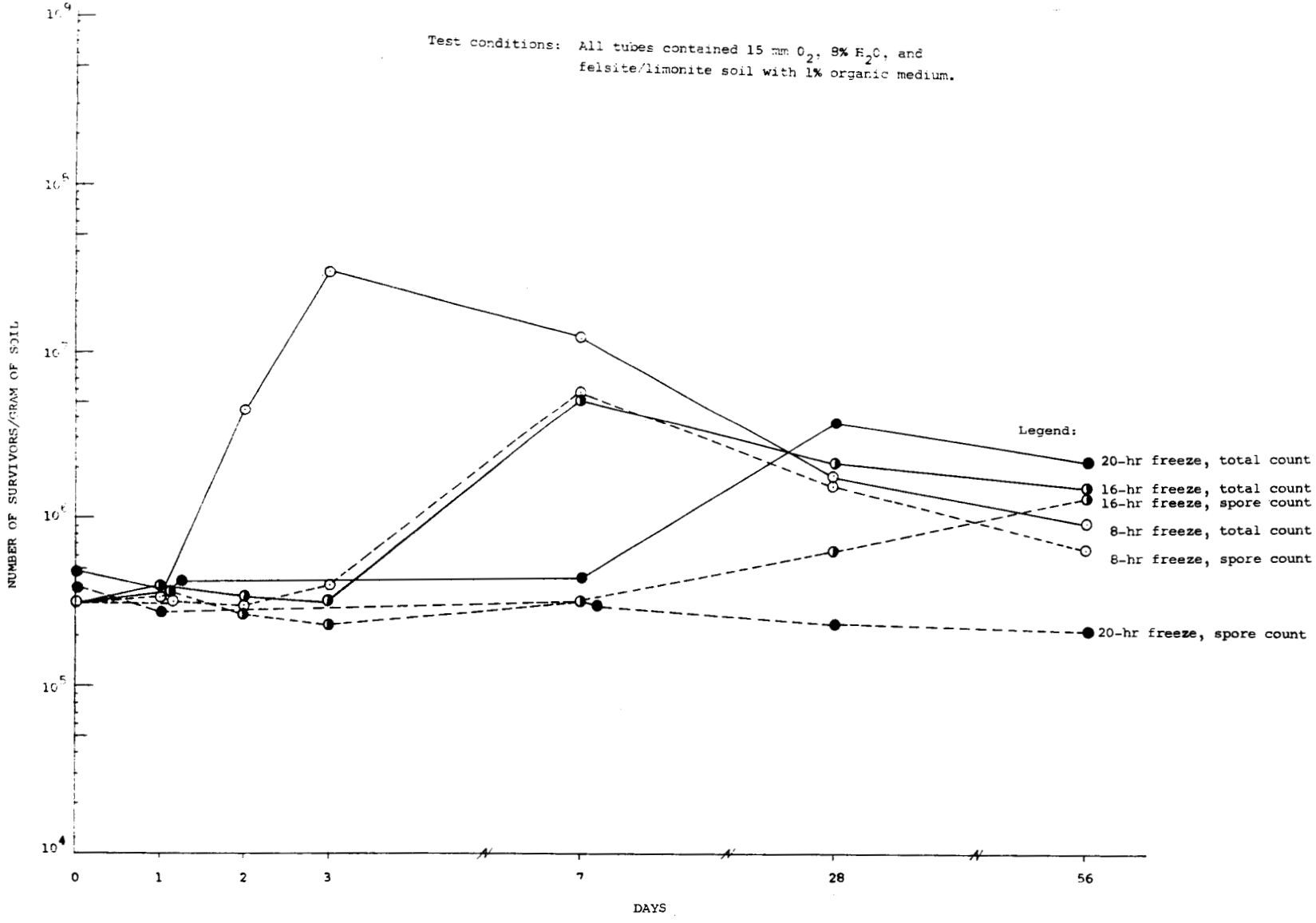

Figure 1: THE EFFECT OF DIFFERENT FREEZE CYCLES ON BACILLUS CEREUS SPORES

It appeared that B. cereus would grow and sporulate in a modified simulated Martian environment with a freeze cycle duration of at least 16 hr and would grow with no sporulation in this environment with a 20-hr freeze cycle. A general trend in growth response of this organism occurred; that is, spore germination followed by vegetative cell growth and sporulation with maximum populations were reached after 7 to 28 days; this was followed by a slight decrease and a final leveling off of the populations. The cell count remained greater than the initial count.

It was of interest to determine whether these cells could reestablish themselves in this environment, considering the possible contamination of an entire planet like Mars where surface winds disturb the soil surface. Transfer Groups 1 and 2 were used to study this possibility. Bacterial cells taken from the Control Group after the logarithmic growth phase were reintroduced into the simulated Martian environment in order to determine whether the cells were able to grow and sporulate.

The results, presented in Figures 2 and 3, indicate that growth did occur. The growth response of Transfer Group 1 (Figure 2) was very similar to that of the Control Group initially studied (Figure 1). Increasing the freeze cycle duration to 16 hr delayed spore germination and vegetative cell growth at least 2 days and sporulation at least 4 days. These findings compare favorably with those from the Control Group. Also, populations significantly higher than the initial

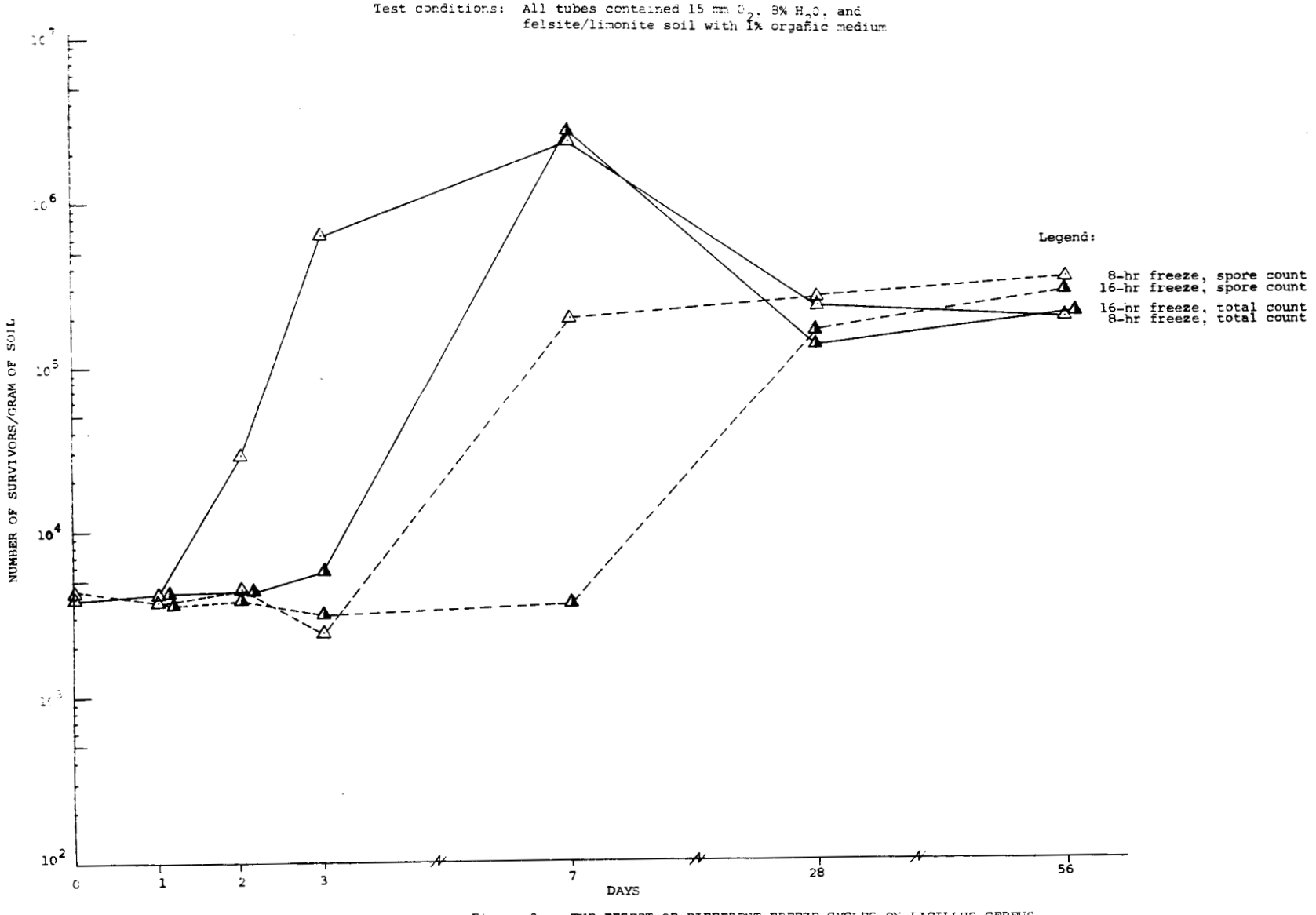

Figure 2: THE EFFECT OF DIFFERENT FREEZE CYCLES ON BACILLUS CEREUS SPORES PRODUCED IN A SIMULATED MARTIAN ENVIRONMENT

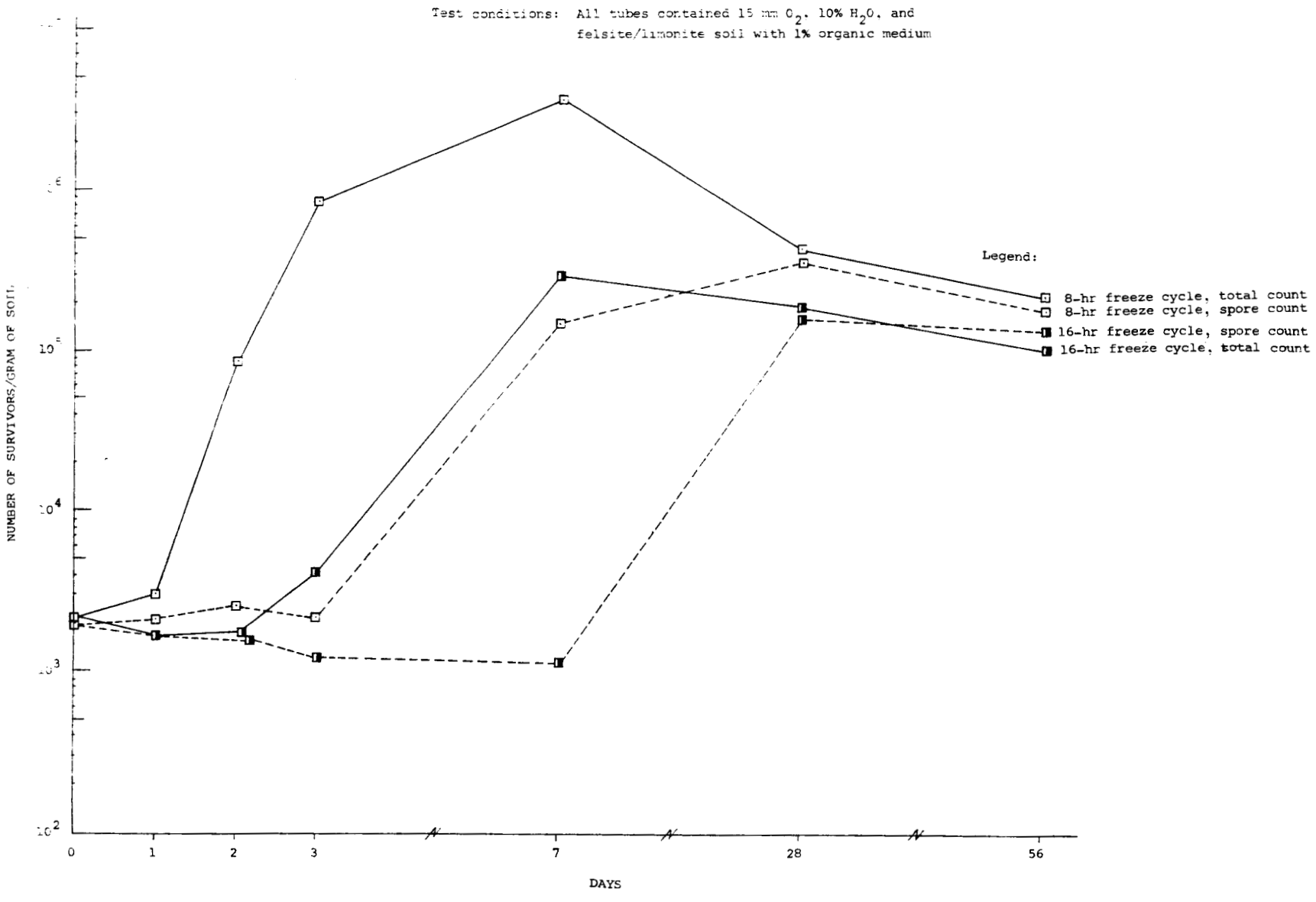

Figure 3: THE EFFECT OF DIFFERENT FREEZE CYCLES ON BACILLUS CEREUS SPORES PRODUCED IN A SIMULATED MARTIAN ENVIRONMENT

populations but lower than maximum were established after 56 days, this was similar to the Control Group.

When this transfer procedure was repeated by using cells derived from Transfer Group 1, similar results were obtained, as indicated in Figure 3. Increasing the freeze cycle to 16 hr delayed spore germination and vegetative cell growth at least 2 days and sporulation at least 4 days.

These studies were extended by using B. subtilis. The tubes were handled in the same manner as for the B. cereus experiments.

The growth response of the B. subtilis Control Group (Figure 4) to an increase in the freeze cycle was comparable with that of B. cereus. Spore germination and vegetative cell growth were delayed at least 1 day, and sporulation was delayed at least 2 days. Increasing the freeze cycle to 20 hr delayed spore germination and vegetative cell growth at least 3 days, and sporulation did not occur during the 56-day period.

The response of B. subtilis Transfer Group 1 and 2 (Figures 5 and 6) was similar to that of the Control Group. With a 16-hr freeze cycle, spore germination and vegetative cell growth were delayed in both Transfer Groups at least 1 day and sporulation at least 2 days. Extension of the freeze cycle to 20 hr delayed spore germination and vegetative cell growth at least 3 days. Sporulation of B. subtilis, although slight, was delayed at least 6 days.

Comparison of the growth responses of B. cereus and B. subtilis showed that B. subtilis growth responses were delayed less and tnat maximum populations were reached sooner than B. cereus.

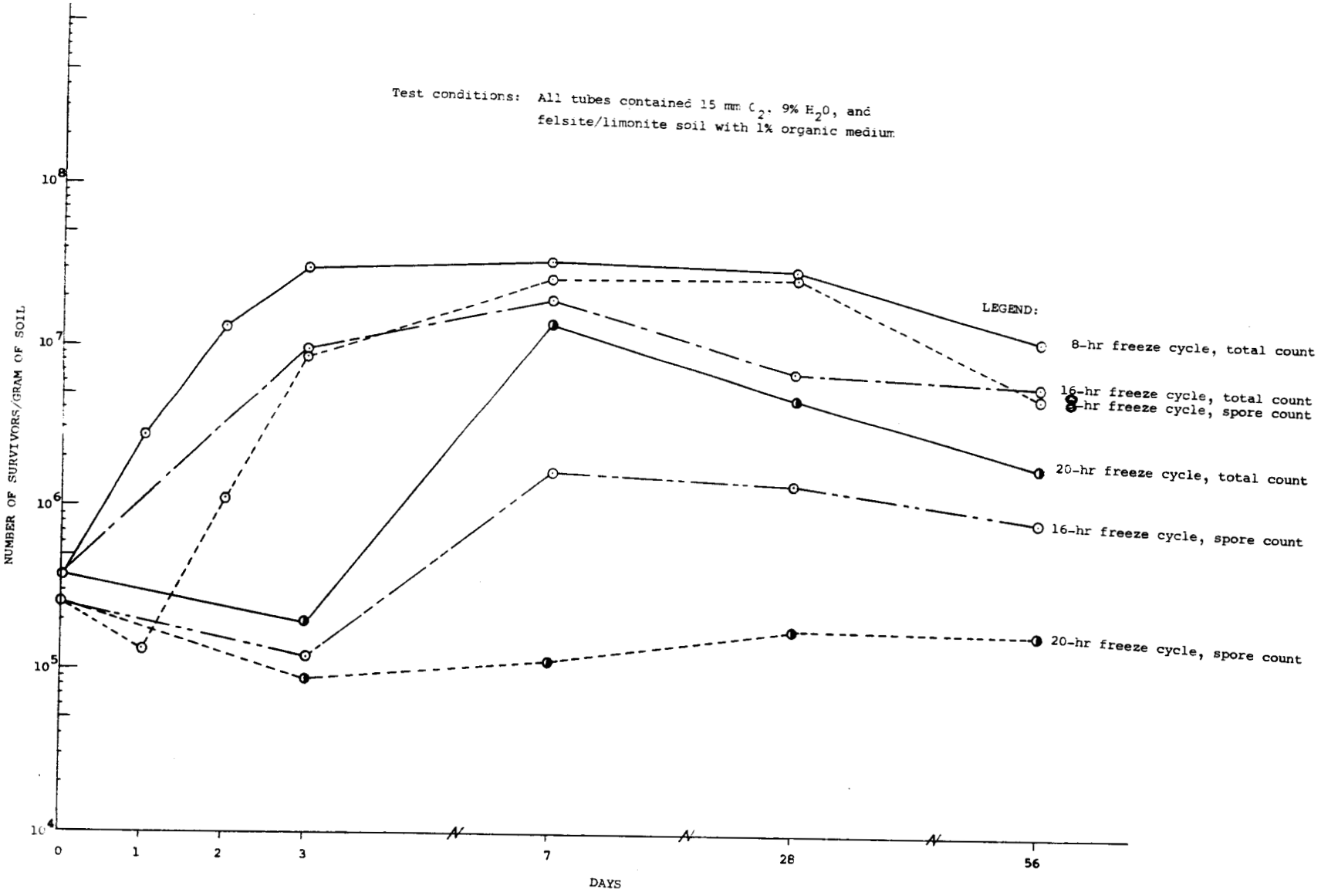

Figure 4: THE EFFECT OF DIFFERENT FREEZE CYCLES ON BACILLUS SUBTILIS SPORES

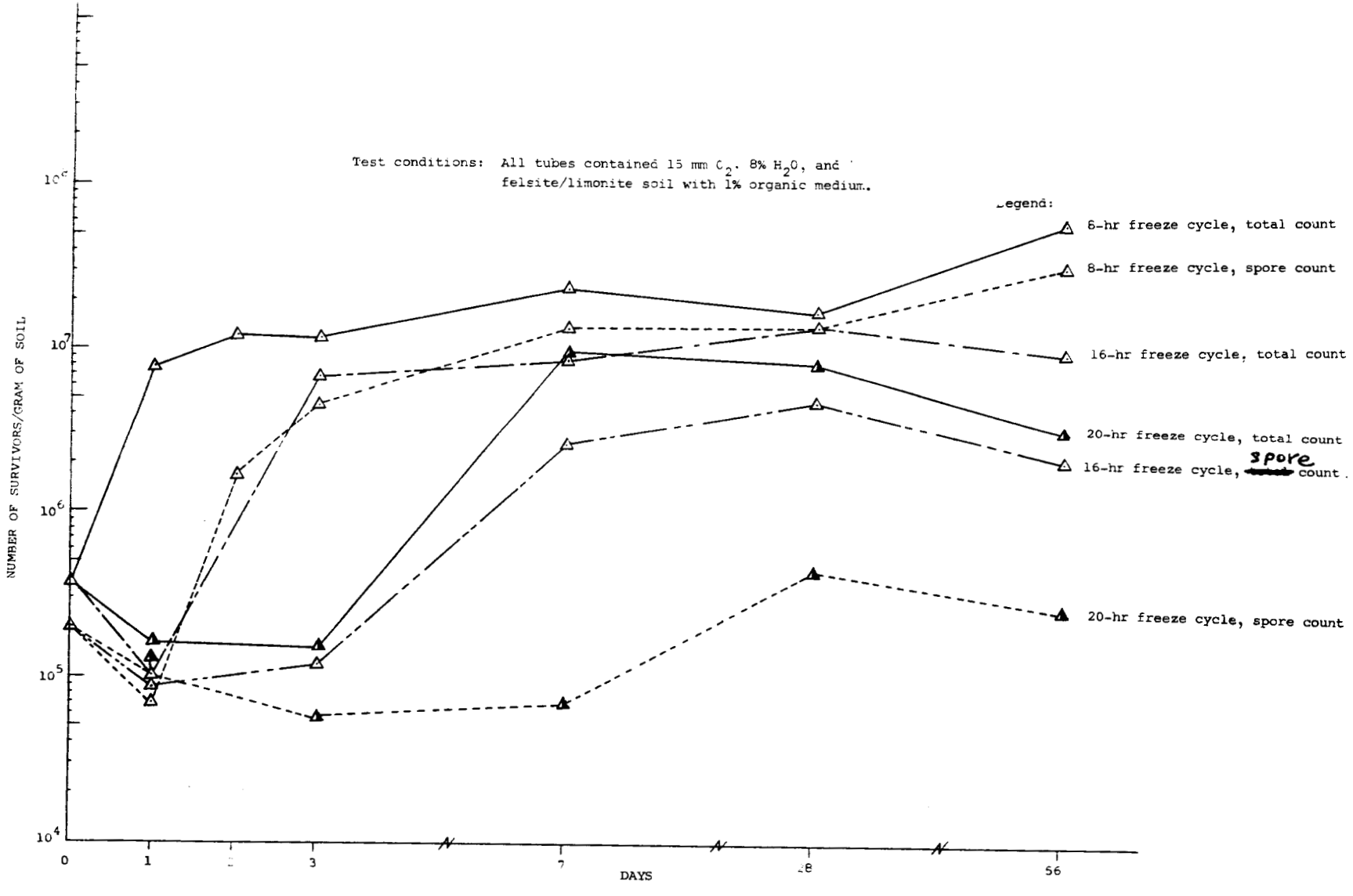

Figure 5: THE EFFECT OF DIFFERENT FREEZE CYCLES ON BACILLUS SUBTILIS SPORES PRODUCED IN A SIMULATED MARTIAN ENVIRONMENT

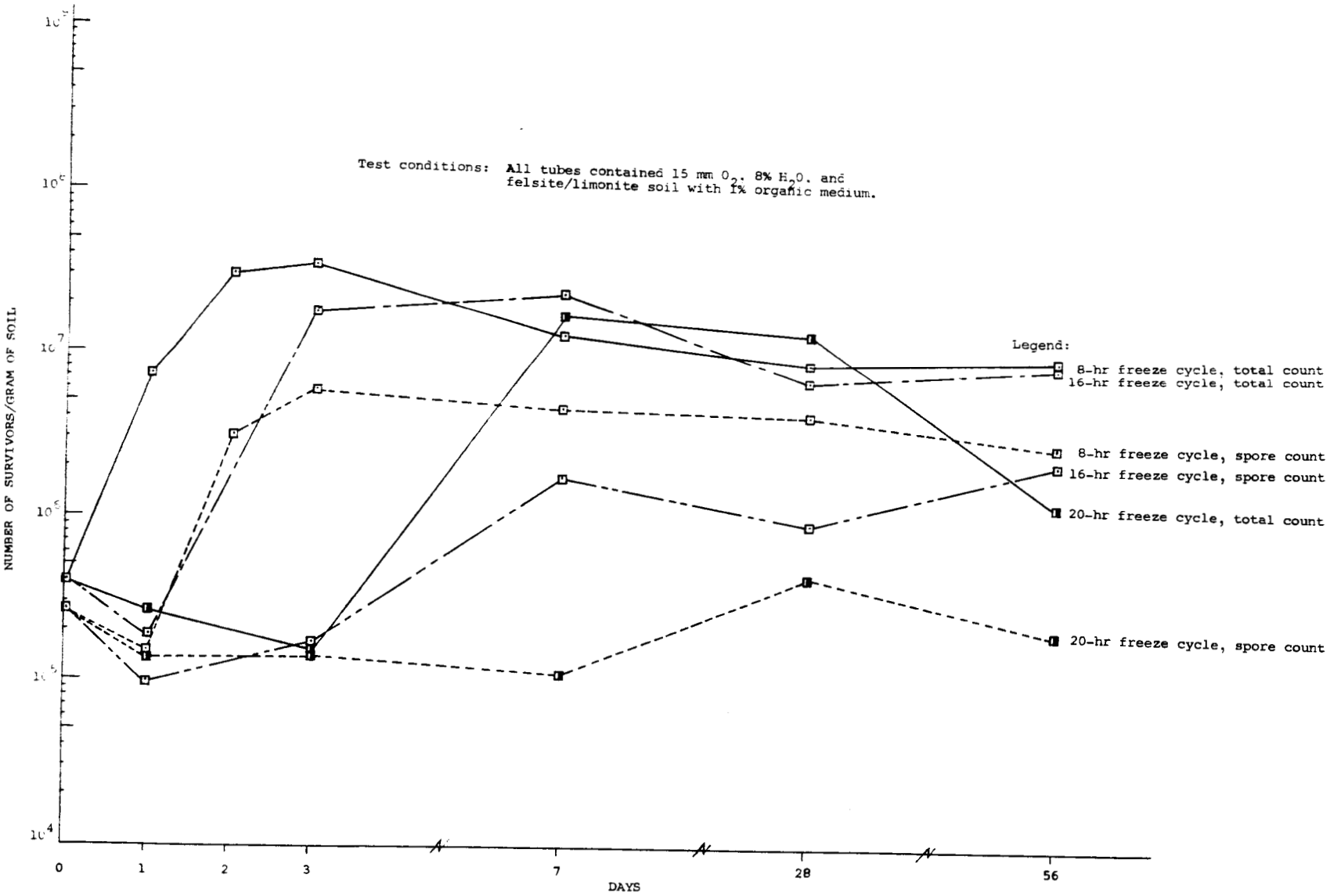

Figure 6: THE EFFECT OF DIFFERENT FREEZE CYCLES ON BACILLUS SUBTILIS SPORES PRODUCED IN A SIMULATED MARTIAN ENVIRONMENT

There were indications that, after 56 days in the simulated Martian environment, the majority of B. cereus cells present were spores, whereas in many instances the majority of B. subtilis cells were vegetative cells. The latter statement is made with reservations since it is difficult to determine accurately the number of spores that germinate without heat-shock.

Projected conclusions from these studies indicate that, if these organism were placed in an similar extraterrestrial environment, contamination and subsequent growth cycles could occur from bacteria growing and being dispersed by winds. Qualifications must be added, however. Moisture is required for the vegetative growth of B. cereus, and moisture with small amounts of oxygen are required for vegetative growth of B. subtilis and sporulation of both organisms.

Other studies were concerned with the possible anaerobic sporulation of B. cereus and vegetative cell growth and sporulation of B. subtilis in the simulated Martian environment by using 2, 0.2, and 0.02% potassium nitrate as a terminal electron acceptor in place of oxygen.

A slightly greater vegetative growth response of B. cereus was noticed with 0.2 and 0.02% nitrate levels (Figure 7). No sporulation was demonstrated. The poorer response at the 2% nitrate level could be related to the lowered water activity of the soil substrate, resulting from increased salt concentration, since the moisture content was at the minimal threshold for growth. The results with B. subtilis, indicated that vegetative cell growth without sporulation occurred at all three nitrate levels

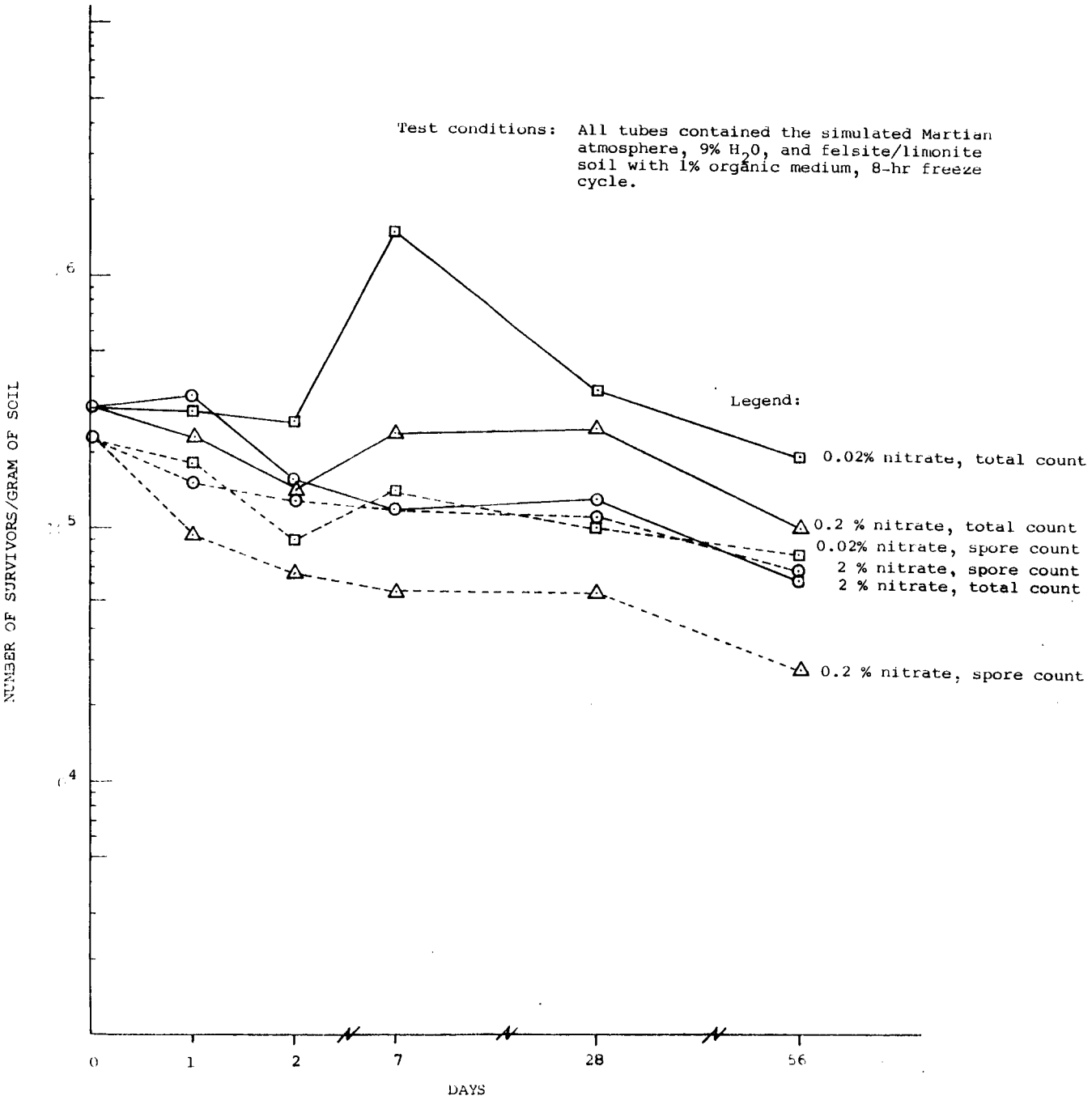

Figure 7: THE EFFECT OF DIFFERENT NITRATE CONCENTRATIONS ON THE ANAEROBIC GROWTH AND SPORULATION OF BACILLUS CEREUS

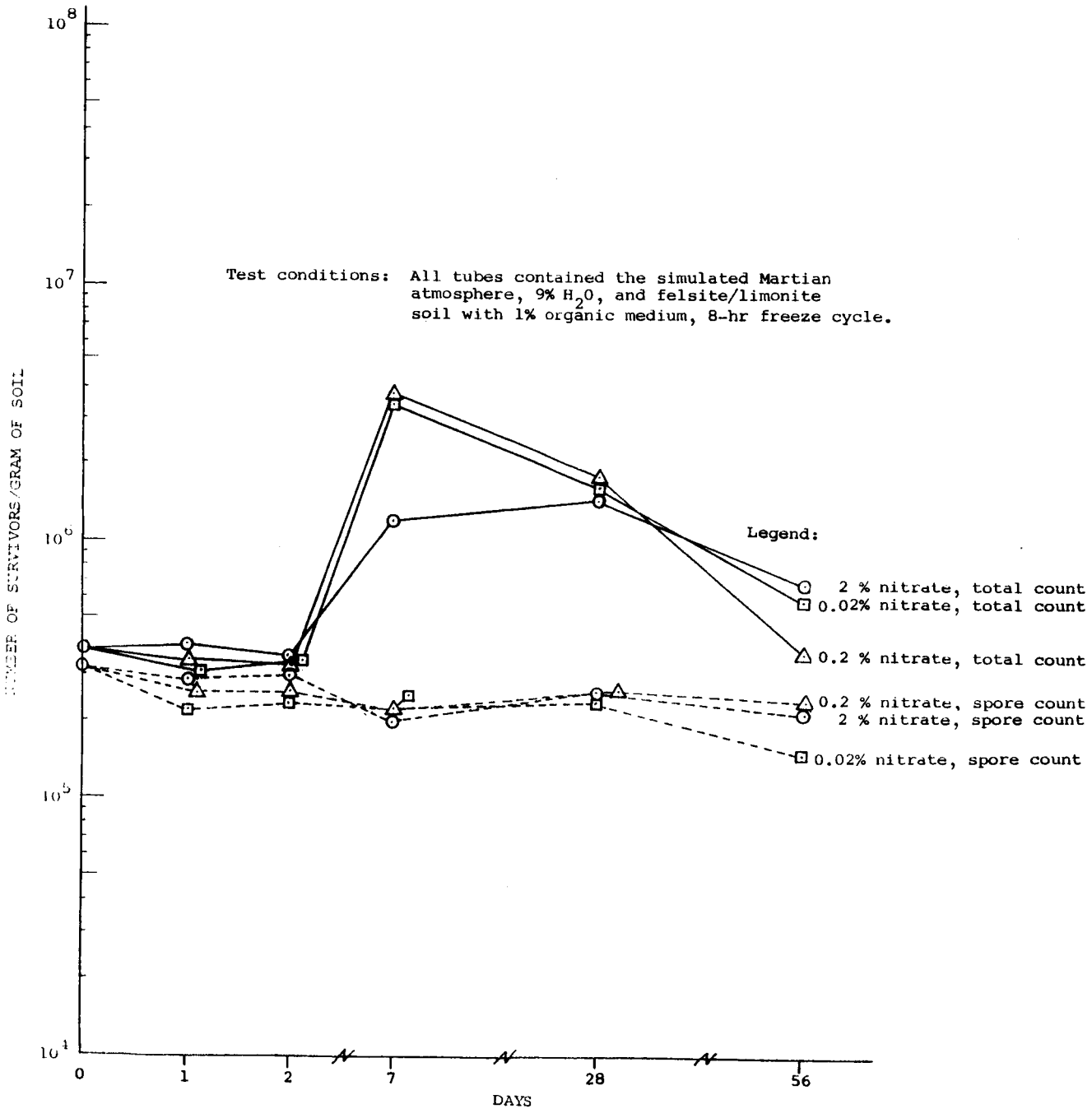

Figure 8: THE EFFECT OF DIFFERENT NITRATE CONCENTRATIONS ON THE ANAEROBIC GROWTH AND SPORULATION OF BACILLUS SUBTILIS

(Figure 8). These experiments were repeated with both B. cereus and B. subtilis by using the transfer method. Concentrations of 0.2 and 0.02% nitrate were used with 8 to 10% moisture. The results, erratic though favorable, indicated that both B. cereus and B. subtilis grew very well but did not sporulate.

IV. SUMMARY

Completed 56-day growth response studies with spores in a modified simulated Martian environment with different freeze cycles indicated that:

(1) Extending the freeze cycle from 8 to 16 hr delayed B. cereus spore germination and subsequent vegetative cell growth 2 days and sporulation at least 4 days. B. subtilis spore germination, vegetative cell growth, and sporulation were delayed at least 2 days.

(2) Extending the freeze cycle from 8 to 20 hr delayed B. cereus spore germination and vegetative cell growth 6 days. B. subtilis spore germination and vegetative cell growth were delayed at least 3 days. Sporulation of either organisms was not apparent during the 56 days.

(3) B. cereus and B. subtilis cells produced in the simulated Martian environment retained their viability and were able to reestablish an ecological niche when transferred into a similar environment.

(4) After 56 days the majority of remaining B. cereus cells were spores; the remaining B. subtilis cells were vegetative cells.

(5) The growth response (vegetative cell growth and sporulation) of B. subtilis was more rapid, and maximum populations were reached before B. cereus.

V. PERSONNEL AND RECORDS

Experiments were planned with the counsel of Dr. E. J. Hawrylewicz and the technical assistance of Miss Charlene Berger, Miss Vivian Tolkacz, and Mr. John Collum.

Experimental data are recorded in Logbooks C15491, C15783, and C15843.

Respectfully submitted,

IIT RESEARCH INSTITUTE

Charles A. Hagen
Associate Bacteriologist
Life Sciences Research

Approved by:

E. J. Hawrylewicz
Assistant Director
Life Sciences Research

CAH/bia

Copy No. _____

Distribution List:

Copy No.	Recipient
1 - 25	Office of Grants and Research Contracts Office of Space Scienc es National Aeronautocs and Space Administration Washington 25, D.C. Attention: SC
26	Dr. Irvin Davis USAF School of Aerospace Medicine Box 4213 Brooks Air Force Base, Texas 78235
27	Dr. C. S. Pittendrigh Department of Biology Princeton University P.O. Box 704 Princeton, New Jersey
28	Dr. Allan Brown Department of Botany University of Minnesota Minneapolis, Minnesota
29	Dr. E. C. Pollard Visiting Professor of Biophysic Pennsylvania State University University Park, Pennsylvania
30	Dr. Norman Horowitz Biology Department California Institute of Technology Pasadena, California
31	Dr. Melvin Calvin Space Sciences Laboratory University of California Berkeley 4, California
32	Dr. Sidney Fox Florida State University Tallahassee, Florida
33	Dr. Carl Sagan Harvard College Observatory Cambridge 38, Massachusetts

Copy No.	Recipient
34	Dr. Dale Smith Ames Research Center National Aeronautics and Space Administration Moffett Field, California
35	Dr. Dale Jenkins Office of Space Sciences National Aeronautics and Space Administration Washington 25, D.C.
36	Dr. R. E. Cameron Jet Propulsion Laboratiries California Institute of Technology Pasadena, California
37	Dr. Carl Bruch Office of Space Sciences National Aeronautics and Space Administration Washington 25, D.C.
38	IIT Research Institute Division L Files
39	IIT Research Institute Editors, Main Files